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Non-Contact Microrheology of Monolayers and Membranes
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Understanding the mechanics/rheology of surface monolayers and lipid bilayers is of fundamental biological importance. One technique used to explored these questions is membrane microrheology, in which the observed thermal fluctuations of a tracer particle in the monolayer is used to extract the rheological data. This technique is challenging for at least two reasons. On the one hand, in fragile monolayer systems the presence of the tracer can locally perturb the monolayer. On the other hand, in sufficiently stiff monolayers/membranes it has proved problematic to embed the particle in it. In this talk, we develop a noncontact microrheological approach to avoid these issues by exploring the effect membrane rheology on the thermal fluctuations of a bead in the fluid near the monolayer.
Specifically, we develop the theory of the force response function of a spherical particle submerged below either a Langmuir monolayer or a lipid bilayer. We show that one can use the observed thermal fluctuations of that submerged particle to extract rheological properties of the essentially two-dimensional monolayer or membrane. We also present experimental results on application of this technique to surfactant monolayers and monolayers bound to an F-actin network on the aqueous side of the Langmuir monolayer.

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Ultrasound-Induced Currents in Planar Lipid Blayers: Origins and Potential Physiological Significance
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Low-intensity focused ultrasound shows great promise for non-invasive, spatially resolved modulation of neural activity *in vivo*. To determine the mechanisms involved in ultrasonic modulation of neural activity and guide the development of this technology, we have been investigating the effects of ultrasound on protein-free planar lipid bilayers. Previously, we reported that ultrasound causes decaying current oscillations in planar bilayers at the onset and offset of the stimulus. These on and off responses are of opposite polarity but otherwise identical. Here, we report that if the rise time of the ultrasound pulse is prolonged, the on response is resolved into two distinct components: a sigmoidal component during the rise time and a damped oscillating component once the pulse reaches its final value. This result suggests that changes in ultrasound intensity during the rise time of the pulse may be important in determining the response to ultrasound *in vivo*, and is consistent with the observation that pulsed ultrasound is more effective than continuous ultrasound in modulating neural activity. To investigate further the origins of the on/off behavior, we used an optical interferometer to measure the velocity of the ultrasound-induced movement (acoustic streaming) in the solution surrounding the bilayer. We find that the time course of the ultrasound-induced current matches the time course of the streaming velocity, with a ratio of 162 pA/(mm/s). This acoustic streaming is probably due to the action of ultrasonic radiation force. To explore the potential physiological relevance of these effects, and to obtain further mechanistic insight, we are investigating the response of planar bilayers to ultrasound under current-clamp. In preliminary experiments, we find that ultrasound pulses with intensity comparable to those used *in vivo* produce voltage changes that would be sufficient to initiate an action potential.

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The Role of the Membrane Confinement for Cell Morphology and Surface Area Regulation
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Commonly, the membranes of mammalian and plant cells are not isolated, but rather they are confined to an actin cortex on the inner side and to an extracellular matrix, cell wall or a substrate on the outer side. The confinement restricts the modes of the membrane deformation and so influences the mechanisms for shape remodeling and surface area regulation. To study the mechanics of confined membranes we developed an in vitro system, which couples a lipid bilayer to the strain-controlled deformation of an elastic sheet (Staykova et al., PNAS 108:9084-9088, 2011). We demonstrate that upon contracting the elastic support, membrane protrusions grow out of the membrane plane, thus reducing its area; upon stretching, the bilayer absorbs the protrusions and expands its area without the lost of integrity. Most of our observations can be understood in terms of free energy minimization in a theoretical model we develop to account for stretch and curvature elasticity of each monolayer, the adhesion with the substrate, and the limited amount of area and volume available to the protrusions. However, some of the observed morphologies are dynamical in nature, and simulations suggest that the friction between the lower monolayer and the substrate plays a key role. Our experimental and theoretical results closely reproduce membrane processes found in, for example, shrinking neurons, and plant and muscle cells, and thus offer a mechanistic approach towards understanding the area regulation in cells.

Platform: Cardiac Electrophysiology

171-Plat
Novel Roles of Fibroblast Growth Factor Homologous Factors in Heart
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Genetic studies of inherited arrhythmias have highlighted essential physiologic roles of modulator proteins that affect ion channel pore-forming subunits. One such class of modulators is the fibroblast growth factor (FGF) homologous factors (FHFs; FGF11-14), that, despite their homology with FGFs, are incapable of functioning as growth factors, are intracellular modulators of Na^+ channels and are associated with neurodegenerative diseases. Unexpectedly, recent work in the Pitt lab has shown that mutations in FHFs affect voltage-gated calcium channels also, indicating that the FHFs can modulate multiple ion channels. The Pitt lab has recently characterized the role of the prominent heart FHF, FGF13, in modulating cardiac Na^+ channel function and trafficking, and further, $FGF13^{X/-}$ mice show a widened QRS and aberrant atrial conduction, therefore we hypothesized that FHFs regulate other ion channels in heart as well, particularly the L-type calcium channel (LTCC, $Ca_v1.2$).
In these studies, we use whole-cell patch clamp to show that in both a heterologous expression system and mouse ventricular cardiomyocytes, FGF13 affects $Ca_v1.2$ current density and kinetics. Immunostaining for the LTCC when FGF13 is knocked down results in aberrant localization of the pore-forming α-subunit, α_{1C}, and changes in T-tubule ultrastructure. Immunocytochemical analysis of adult mouse cardiomyocytes shows that FGF13 has a broad cellular distribution. Interestingly, a portion of the population colocalizes with Ankyrin B, a protein responsible for trafficking multiple ion channels in the heart.
These data of FHF's novel function in heart begin to define FHF's role in excitation-contraction coupling and cardiac arrhythmogenesis. These cellular mechanisms will lay the groundwork for in vivo electrophysiology studies on $FGF13^{X/-}$ mice.

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Illuminating Trafficking of KCNQ1/KCNE1 Channels in Heart
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In human heart, pore-forming KCNQ1 subunits associate with auxiliary KCNE1 subunits to generate the slowly activating, delayed rectifier potassium current, I_{Ks}. Decreases in I_{Ks}, due to either congenital mutations in KCNQ1/KCNE1 subunits or the adverse neuro-hormonal milieu accompanying heart failure, delay cardiac repolarization, leading to long QT syndrome (LQTS). LQTS predisposes to lethal ventricular arrhythmias and sudden cardiac death. How KCNQ1/KCNE1 channel trafficking in the heart proceeds and is regulated is unknown. However, understanding this process is critical both for fundamental mechanistic insights into LQTS, and for rational development of potential new therapies for this condition. We sought to develop optical tools that permit direct visualization of KCNQ1/KCNE1 subunit assembly and trafficking in heart cells. KCNQ1 and KCNE1 were tagged intra-cellularly with YFP and CFP, respectively, and extra-cellularly with a 13-residue high affinity α-bungarotoxin (BTX) binding site (BBS). The tagged proteins were viable as determined by functional electrophysiological assays in Chinese hamster ovary (CHO) cells– BBS-KCNQ1-YFP expressed alone gave rise to rapidly-activating outward currents, and co-expression with KCNE1-CFP resulted in the slowly activating kinetic signature of I_{Ks}. Cell surface BBS-tagged channel subunits were selectively detected with quantum dot. Optical pulse chase assays in human embryonic kidney (HEK 293) cells revealed that surface KCNQ1 channels undergo rapid endocytosis and recycling, and this process is largely unaffected by KCNE1. Tagged KCNQ1/KCNE1 subunits expressed in guinea pig heart cells using adenovirus were targeted to the surface sarcolemma, t-tubules and intercalated disks, similar to endogenous channels.

Surprisingly, I_{Ks} amplitude remained unchanged with over-expression of either KCNQ1 or KCNE1, suggesting a tightly regulated system for maintaining KCNQ1/KCNE1 surface density.

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A Previously Unrecognized Conductance is a Critical New Player in the Pacemaker of Cardiomyocytes Derived from Human Embryonic Stem Cells

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The timely appearance and proper functioning of pacemaker activity is a critical feature of heart physiology. Two main mechanisms have been proposed: (1) The ''voltage-clock'', where the hyperpolarization-activated funny current If causes diastolic depolarization that triggers action potential cycling; (2) The ''Ca2+ clock'', where cyclical release of Ca2+ from Ca2+ stores depolarizes the membrane during diastole via activation of the Na+-Ca2+ exchanger (NCX). However, these pacemaker mechanisms remain highly controversial. Here, we used human embryonic stem cell-derived cardiomyocytes (hESC-CMs) to study the embryonic pacemaker mechanisms of the human heart. Combined current- and voltage-clamp recording from the same hESC-CM and blocking If with zatebradine or ZD7288 and NCX with KB-R7943 or FRCRCF peptide revealed distinct pacemaker phenotypes. Results showed that the ''voltage clock'' and ''Ca2+ clock'' pacemakers can coexist in the same cell, but can also occur in a mutually exclusive fashion in other cell populations. Interestingly, all these pacemaker phenotypes shared a depolarizing drift of the maximal diastolic potential (MDP) following exposure of cells to blockers of the ''voltage'' and ''Ca2+ clocks'', suggesting that both mechanisms converge to a common pacemaking component. This MDP depolarization arises from inhibition of a previously unrecognized conductance in hESC-CMs. Remarkably, blockade of this conductance leads to depolarization of the MDP and suppresses pacemaker activity. Data are discussed on how this conductance plays a crucial role in human embryonic cardiac automaticity.

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Real-Time Human and Guinea Pig Action Potentials Anthropomorphized from Neonatal Mouse Cardiomyocytes

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The murine cardiac action potential waveform can be anthropomorphized into that of a human-like waveform in real time, through a novel dynamic-clamp method known as the cell-type transforming clamp (CTC). In the CTC, a computationally calculated virtual conductance is inserted into the cell in real time, to compensate for the differences between murine and human sarcolemmal currents. By so doing, the CTC anthropomorphizes the membrane potential without clamping it, thereby enabling the investigation of drug- or mutation-induced arrhythmogenic phenotypes in the appropriate human action potential context (but in the experimentally powerful mouse animal model).

We are using a real-time implementation of a genetic algorithm that optimizes the morphology of a theoretical model, in order to match the murine action potential recorded from a real cell. We present a comparison of human and guinea pig action potentials anthropomorphized in real time, from neonatal mouse cardiomyocytes.

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Estrogen Therapy Abolishes Spontaneous Ventricular Arrhythmias in Right Ventricular Failure Induced by Pulmonary Hypertension

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We previously have shown that pulmonary hypertension (PH)-induced right ventricular failure (RVF) is associated with increased incidence of sudden death caused by spontaneous ventricular fibrillation (VF). We also discovered that estrogen (E2) therapy rescues severe PH and RVF and results in 100% survival. Here we hypothesized that E2 abolishes spontaneous VF associated with RVF by restoring RVEF, reversing fibrosis and restoring PH-induced downregulation of repolarizing K-channel proteins Kv1.5 and KCNE-2 and SERCA-2a expression. Chronic PH-associated RVF was induced in male rats by s.c. monocrotaline (MCT, 60 mg/kg, n=10). Some MCT-rats were treated with E2 (42.5 ug/kg/day, s.c.) from day 21 (severe PH-stage) to day-30 (n=8). Saline treated rats served as control (n=8). At ~day 30, hearts were studied in isolated-perfused Langendorff setting. RV-epicardial activation pattern was optically mapped using fluorescent voltage-sensitive dye (RH-237). By ~day 30, 30% RVF rats died suddenly but none in the control or E2-groups. RVF hearts manifested EADs and spontaneous VF during normal Tyrode's perfusion with wavefront dynamics supported by both focal and multiple wavelet patterns. No VF was initiated in any of the control or E2-treated hearts. SERCA-2a was reduced (~15-fold) in RVF (0.06 ± 0.01 vs. 1 ± 0.26 control) that was reversed in E2-group (0.77 ± 0.13, $p<0.05$ vs. RVF). Kv1.5 was reduced ~8 fold in RVF (0.12 ± 0.03 vs. 1 ± 0.18 in control, $p<0.05$) and E2 partially restored Kv1.5 (0.46 ± 0.06, $p<0.05$). KCNE2, an ancillary K-channel subunit, was reduced ~3-fold in RVF (0.3 ± 0.1 vs. 1 ± 0.1, $p<0.05$) that was reversed by E2 (0.9 ± 0.05, $p<0.05$). RVF was associated with moderate RV-fibrosis and severe reduction in RV-ejection fraction (RVEF) from 65 ± 1 to $33 \pm 3\%$. E2- group did not show any fibrosis and RVEF was preserved ($60 \pm 2\%$). In conclusion, E2-therapy rescues RVF and prevents VF by reversing PH-induced RV-fibrosis and reduced repolarization-reserve.

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Anisotropic Biaxial Stretch Slows Longitudinal and Transverse Conduction in Micropatterned Mouse Ventricular Myocyte Cultures

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The effects of stretch on cardiac conduction velocity are controversial, and several counteracting mechanisms have been proposed. Conflicting reports of conduction velocity increase and decrease under cardiac loading have been reported. These changes have been attributed to stretch modulation of ion channels, cell-cell junctions, cell capacitance, and properties of the interstitium. To separate the effects of tissue geometrical changes from intrinsic changes in myocyte conduction and to eliminate effects of stretch on interstitial electrical properties, neonatal murine cardiomyocytes were cultured on micropatterned stretchable substrates for optical mapping of excitation conduction velocity. A homogeneous anisotropic biaxial strain field of 14% in the primary direction and 3.6% in the secondary direction was applied to these substrates, where the primary direction of stretch was oriented either parallel or perpendicular to the longitudinal axis of the aligned cell culture. When the primary direction of strain was oriented parallel to the longitudinal cell culture axis, the longitudinal conduction velocity slowed to 72% ± 3% and recovered to 95% ± 14% of baseline conduction following unloading, while the transverse conduction velocity slowed to 75% ± 3% and recovered to 137% ± 19% (n=3, mean ± SEM). When the primary direction of strain was oriented along the transverse cell culture axis, the transverse conduction velocity slowed to 77% ± 6%, while the longitudinal conduction velocity slowed to 84% ± 9%, neither recovering following unloading (n=3, mean ± SEM). Conduction velocity slowed in both longitudinal and transverse directions under biaxial strain and substantially recovered following unloading in the case of primarily axial stretch.

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A Novel Role for Ephaptic Coupling in Cardiac Conduction: An Experimental and Modeling Study

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Introduction: We demonstrated that edema slows conduction (θ and increases anisotropy (AR_θ). Existing mathematical models incorporating only gap junctional (Gj) coupling cannot explain these data. We hypothesized that inhibiting ion channels during edema would unmask ephaptic coupling effects.

Methods: θ and AR_θ were quantified by optical mapping in Langendorff-perfused guinea pig ventricles. Mannitol (26.1g/l) was perfused to increase VIS. INa, which partially colocalizes with Gj, was inhibited by flecainide (0.5μM). IK1, which exhibits low intercalated disk colocalization with Gj, was inhibited by $BaCl_2$ (10 μM). Conduction was mathematically modeled in a 2D tissue slab with both Gj and ephaptic coupling and appropriate INa and IK1 cellular distributions.

Results: During control, longitudinal (θ_L) and transverse (θ_T) θ were 51.9 ± 1.0cm/s and 21.2 ± 0.6cm/s respectively; AR_θ was 2.5 ± 0.1. Flecainide decreased both θ_L and θ_T by $17 \pm 2\%$ (n=5, $p<0.05$ vs. control). $BaCl_2$ increased both θ_L and θ_T by $23 \pm 2\%$ (n=4, $p<0.05$ vs. control). Neither altered AR_θ (p=ns vs. control). Mannitol decreased θ_L ($16 \pm 3\%$, n=3, $p<0.05$) and θ_T ($26 \pm 2\%$, n=3, $p<0.05$) and increased AR_θ to 3.0 ± 0.1